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(Nasa CR-53601)

Quarterly Report No. 3, *

RESEARCH IN PHOTOSYNTHESIS

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RIAS Div.
~~9212 Bellona Avenue~~
Baltimore ~~12~~, Maryland

Martin Co.
14820 33

(NASA Contract No. NASw-747)

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* December 6, 1963 - March 6, 1964

Principal Investigator - Bessel Kok [1964] 6p ozy

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National Aeronautics and Space Administration

(NASA Contract NASw-747)

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Research in Photosynthesis

Chloroplast lipids

Phospholipid and glyceride synthesis from C^{14}-1,3 glycerol-phosphate and C^{14}-1,3 glycerol in isolated chloroplasts and "grana" has been studied. Centrifugal fractionation of spinach homogenates prepared in an isotonic medium shows that the "grana" fraction has the highest specific activity for incorporation of C^{14}-1,3 glycerol-phosphate into lipid. Grana prepared from chloroplasts, however, have a much lower specific activity and thus, it would appear that mitochondria are primarily responsible for the lipid synthesis reported here by the "grana" fraction.

With C^{14}-1,3 glycerol phosphate as substrate, the following co-factors are required for maximal incorporation of substrate into lipids: ATP, Mg, CoASH, and a long chain fatty acid. No specificity for fatty acid has been observed. Comparisons of glycerol phosphate and glycerol as substrates reveal that the rate is approximately 100 times greater with glycerol phosphate than with glycerol. Addition of glycerol kinase eliminates the difference in rates with these substrates thus indicating that this kinase is rate-limiting when glycerol is used.

Products of the reaction have been studied kinetically using ion-exchange and paper chromatography for identification of deacylated products. Results indicate that phosphatidic acid is the precursor for the other

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lipid products. Phosphatidic acid has been identified by its mobility on silicic acid impregnated paper, co-chromatography of its deacylate with glycerol phosphate on an ion-exchange resin and on paper with two different solvent systems. Possibility of it being a bis-phosphatidic acid has been excluded with studies of products resulting from periodate oxidation.

In addition to phosphatidic acid, other phosphatides as well as glycerides are formed. Silicic acid column chromatography of the glycerides indicates that mono-, di-, and triglycerides are formed in varying amounts depending upon duration of assay. The results described here can be summarized in the following scheme:

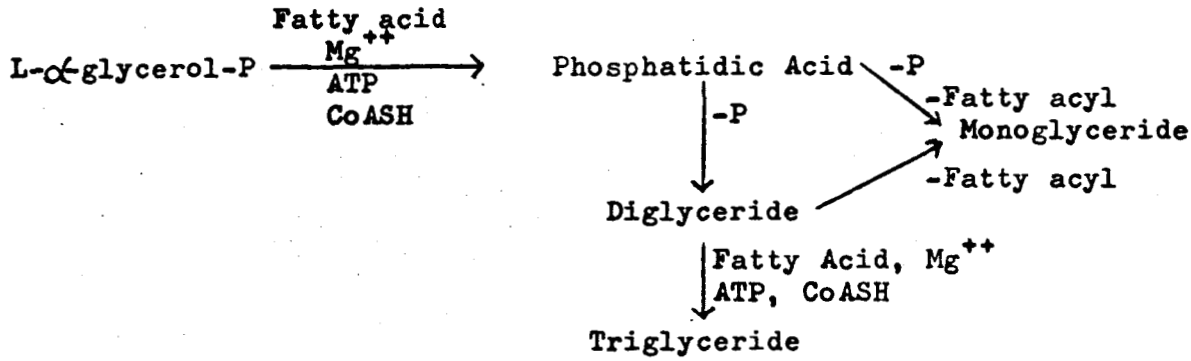

Photo-inhibition

We have tried to evaluate and expand our earlier obtained data on the subject of photo-inhibition. One difficulty encountered was the variability of the results obtained when we studied the photo-inhibition of the chloroplasts' capacity to reduce TPN using ascorbate as electron donor. A seasonal variation of our source of chloroplasts must have been involved. The best interpretation seems that this variation does not concern the susceptibility to strong light as such, but rather the

ability of reduced indophenol to reactivate the reduction of TPN. This leads to our present conclusion that of the two photoreactions of photosynthesis, the one which evolves oxygen is the one most sensitive to inhibition by light. However, also the first photoact, (the one that produces the strong production) is inhibited but with a three-fold lower rate constant. A paper reporting on these matters is presently in preparation. The next phase of this work will concern a study of the activation spectrum of photo-inhibition. We will try to illucidate whether chlorophyll is the sole sensitizing pigment or other pigments absorbing blue or ultra-violent light contribute in an independent fashion. This will test our present working hypothesis that photo-inhibition is due to an excessive rate of photosynthetic photochemistry and a damaging reaction of initial photoproducts which cannot be removed fast enough. To obtain sufficiently high intensities of monochromatic light, a micropolarographic method for measuring rates of oxygen is presently being developed.

Photosynthetic electron transport

Of the several lines of research presently being pursued, we may mention the following: it concerns the redox potential of the primary photoreductant made in photosynthesis. The initial mechanism of CO_2 reduction in complete photosynthesis is not fully understood. There are doubts whether TPNH and ATP are really the first stable products of photosynthesis, which in turn reduce CO_2 to sugar. If the primary photoreductant has a low enough potential, a direct photoreduction of an acid to aldehyde were thermodynamically possible. A second aspect of this problem concerns the site of photophosphorylation. In chloroplasts the reduction of TPN is "coupled" to the formation of ATP. One explains this by assuming that

electrons move towards TPN via a reaction step which generates ATP from ADP. Theoretically, the energy available in the red light quantum would allow the formation of a reductant with a potential lower than -1 volt -- since the primary photo-oxidant $P700^+$ has a potential of +.44 volts, Electrons donated to TPN by such a low potential reductant could thus generate ATP. It was shown by other workers that the catalysis of TPN reduction by the enzyme ferridoxin rests upon the fact that ferridoxin, which has a normal reductant of -.42 volt, is first reduced by the primary reductant. Earlier, it was shown in our group that chloroplasts are capable of reducing methyl viologen which has a potential of -.44 volt. Recently we obtained a sample of a viologen derivative with a potential of -.55 volt and we found that this compound was reduced with about equal rate and to about equal degree as methyl viologen. The data strongly indicate the possibility that the primary reductant has a potential of -.5 to -.6 volts or lower.

Oxygen exchanges in chloroplast reactions

The photo-oxidation of ascorbic acid and certain reduced quinones by chloroplasts is a seemingly anomalous reaction in that it is inhibited by herbicides which are specific inhibitors of the oxygen evolution reactions in photosynthesis. The indophenol mediated photo-oxidation of ascorbic acid by chloroplasts has been shown to be coupled to photophosphorylation. Addition of the oxygen evolution inhibitor, DCMU destroys the phosphorylation but does not change the overall rate of oxygen consumption. This photoreaction has been investigated with the mass spectrometer and shown to consist of not solely oxygen consumption but oxygen production which is overcome by a high rate of oxygen uptake. Addition of DCMU abolishes the

oxygen production and diminishes the rate of oxygen uptake. The net oxygen exchange is, in agreement with previous results, not changed by DCMU. This phenomenon presents a means of studying the locus of injection of electrons from external donors into the electron transport chain of photosynthesis.

Photopotentiation of ATP hydrolysis

ATPase activity can be developed in chloroplasts by their illumination in the presence of a sulfhydryl-compound and a redox co-factor. The activity so developed bears many striking similarities to electron transport reactions in chloroplasts. A study has been made of the effect of various inhibitors and activators of the Hill reactions and photosynthetic phosphorylation on this ATPase. All "uncouplers" of photosynthetic phosphorylation at higher concentrations inhibit this ATPase as they do the Hill reaction. Some "uncouplers" at lower concentrations stimulate the ATPase as they also do other Hill reactions. Some compounds which are inhibitors of electron transport and not uncouplers of photosynthetic phosphorylation will stimulate the ATPase. It is hoped that these results will lead to a greater understanding of the mechanism by which ATP formation is coupled to electron transport in photosynthesis.